VSP HOMOLOGIES

TO FIG. 1B.

FIG. 1A.

FROM FIG. 1A.

FIG. 1B.

Title: Compositions and Methods for Altering Amino Acid Content of Proteins
Inventor(s): Rao et al.
Application No: 09/478,598
Atty Dkt N : 5718-16A (35718/193734)

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PROPOSED VSPβ METHIONINE-ENRICHED VARIANTS

	1	5	10	15	20	25	30
VSPβ	R S S E V	K C A S F	R L A V E	A H N I	R A F K T	I I P E E	C V
VSPβ-Met10	M				M		M
VSPβ-Met20	M				M		M
VSPβ-Met30	M				M		M M

	31	35	40	45	50	55	60
VSPβ	E P	T K D Y I	N G E Q F	R S D S K	T D N Q Q	A F F Y A	S E R
VSPβ-Met10		M		M			M
VSPβ-Met20	M	M		M			M
VSPβ-Met30	M	M		M	M		M

	61	65	70	75	80	85	90
VSPβ	E V	H H N D I	F I F G I	D N T V L	S N I P Y	Y E K H G	Y G V
VSPβ-Met10	M	M	M	M			
VSPβ-Met20	M	M M	M	M			M
VSPβ-Met30	M	M M	M	M	M		M

	91	95	100	105	110	115	120
VSPβ	E E	F N E T L	Y D E W V	N K G D A	P A L P E	T L K N Y	N K L
VSPβ-Met10							
VSPβ-Met20		M	M				
VSPβ-Met30	M	M	M				

	121	125	130	135	140	145	150
VSPβ	L S	L G F K I	V F L S G	R Y L D K	M A V T E	A N L K K	A G F
VSPβ-Met10	M		M		M		M
VSPβ-Met20	M		M M	M	M		M M
VSPβ-Met30	M		M M	M	M	M	M M

	151	155	160	165	170	175	180
VSPβ	H T	W E Q L I	L K D P H	L I T P N	A L S Y K	S A M R E	N L L
VSPβ-Met10						M	M
VSPβ-Met20				M	M	M	M
VSPβ-Met30			M	M M	M	M	M

	181	185	190	195	200	205	210
VSPβ	R Q	G Y R I V	G I I G D	Q W S D L	L G D H R	G E S R T	F K L
VSPβ-Met10			M		M		
VSPβ-Met20			M		M M	M	
VSPβ-Met30			M		M M	M	

	211	215	218
VSPβ	P N P	M Y Y I	E
VSPβ-Met10	M	M	
VSPβ-Met20	M	M	
VSPβ-Met30	M	M	

FIG. 2.

HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPB.
TOTAL NUMBER OF AMINO ACIDS IS: 218.

HYDROPATHIC INDEX OF VSPB FROM AMINO ACID 1 TO AMINO ACID 218.
COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=-4.95).

FIG. 3A.

Title: Compositions and Methods for Altering Amino Acid Content of
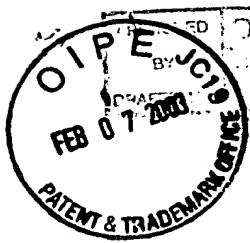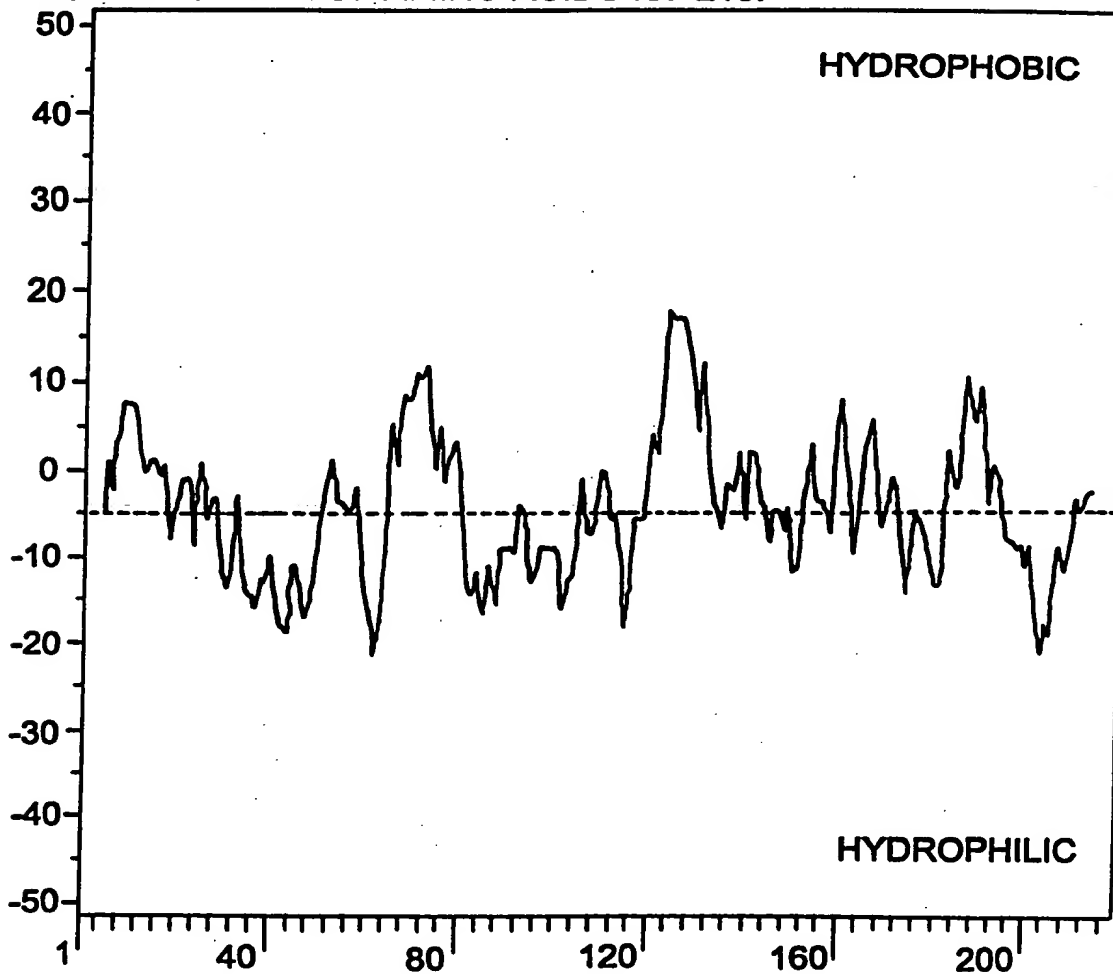Pr...
Inventor(s): Rao *et al.*
Application No: 09/478,598
Atty Dkt No: 5718-16A (35718/193734)

HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPM10.
TOTAL NUMBER OF AMINO ACIDS IS: 218

HYDROPATHIC INDEX OF VSPM1 FROM AMINO ACID 1 TO AMINO ACID 218.
COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=5.52).

FIG. 3B.

HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPM20. TOTAL NUMBER OF AMINO ACIDS IS: 218.

HYDROPATHIC INDEX OF VSPM20 FROM AMINO ACID 1 AMINO ACID 210. COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=-5.68).

FIG. 3C.

Title: Compositions and Methods for Altering Amino Acid Content of
Proteins
Inventor(s): Rao et al.
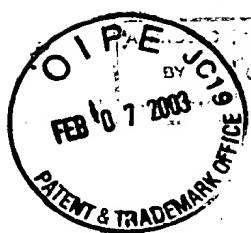Application No: 09/478,598
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**HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPM30.
TOTAL NUMBER OF AMINO ACIDS IS: 218.**

HYDROPATHIC INDEX OF VSPM30 FROM AMINO ACID 1 TO AMINO ACID 218.
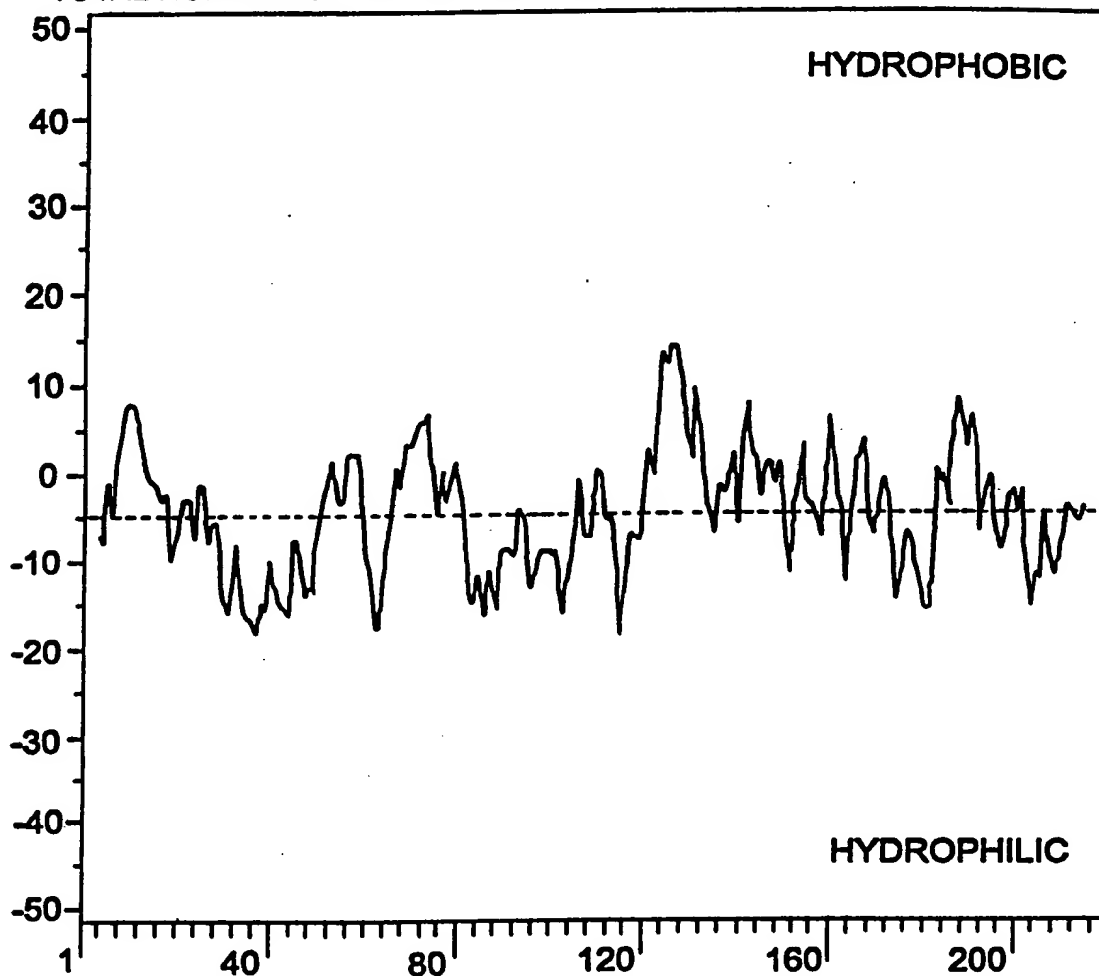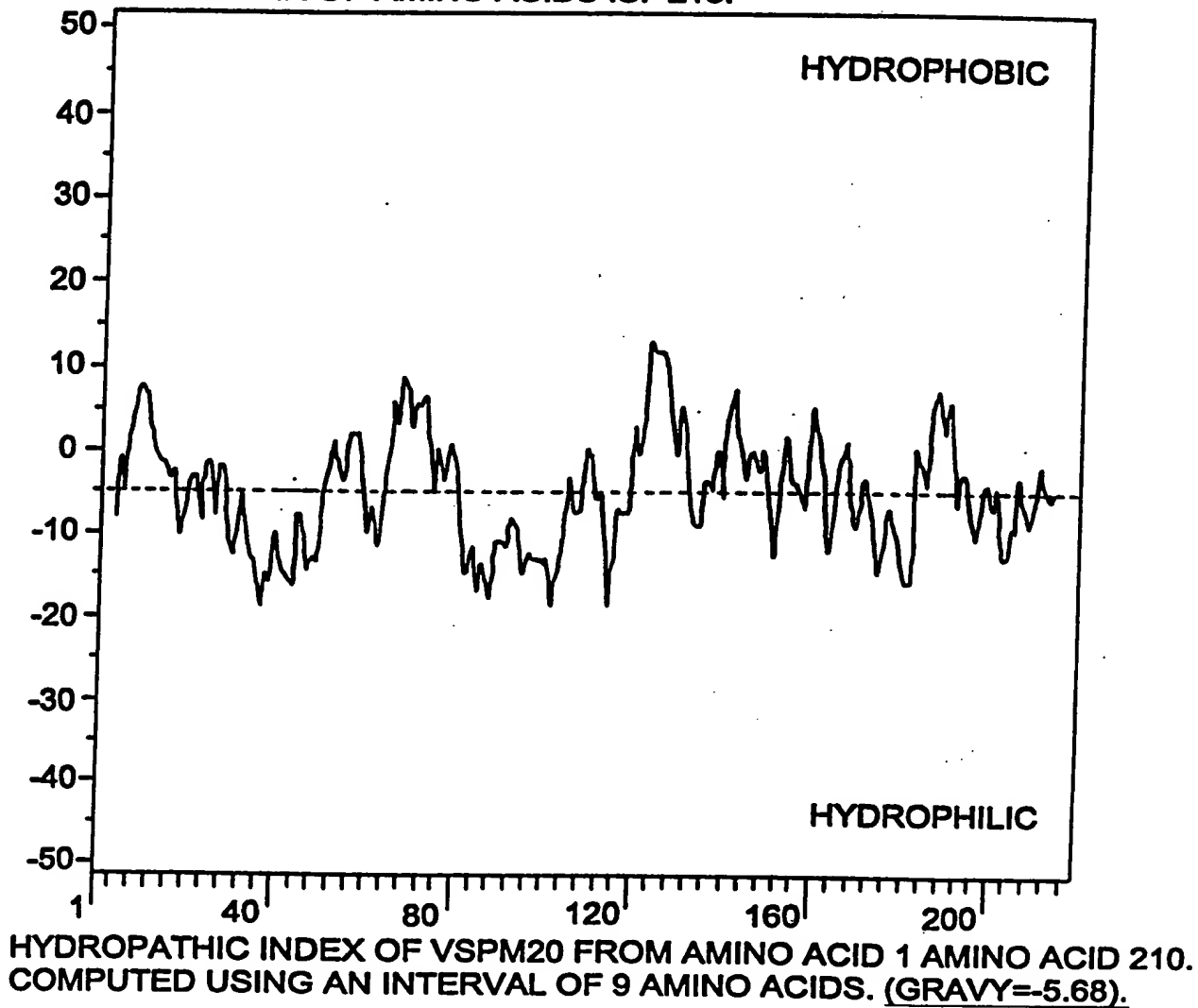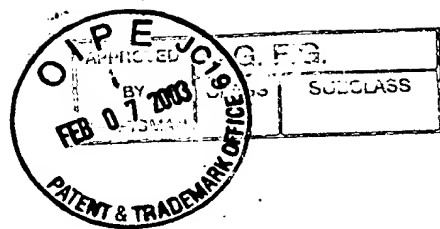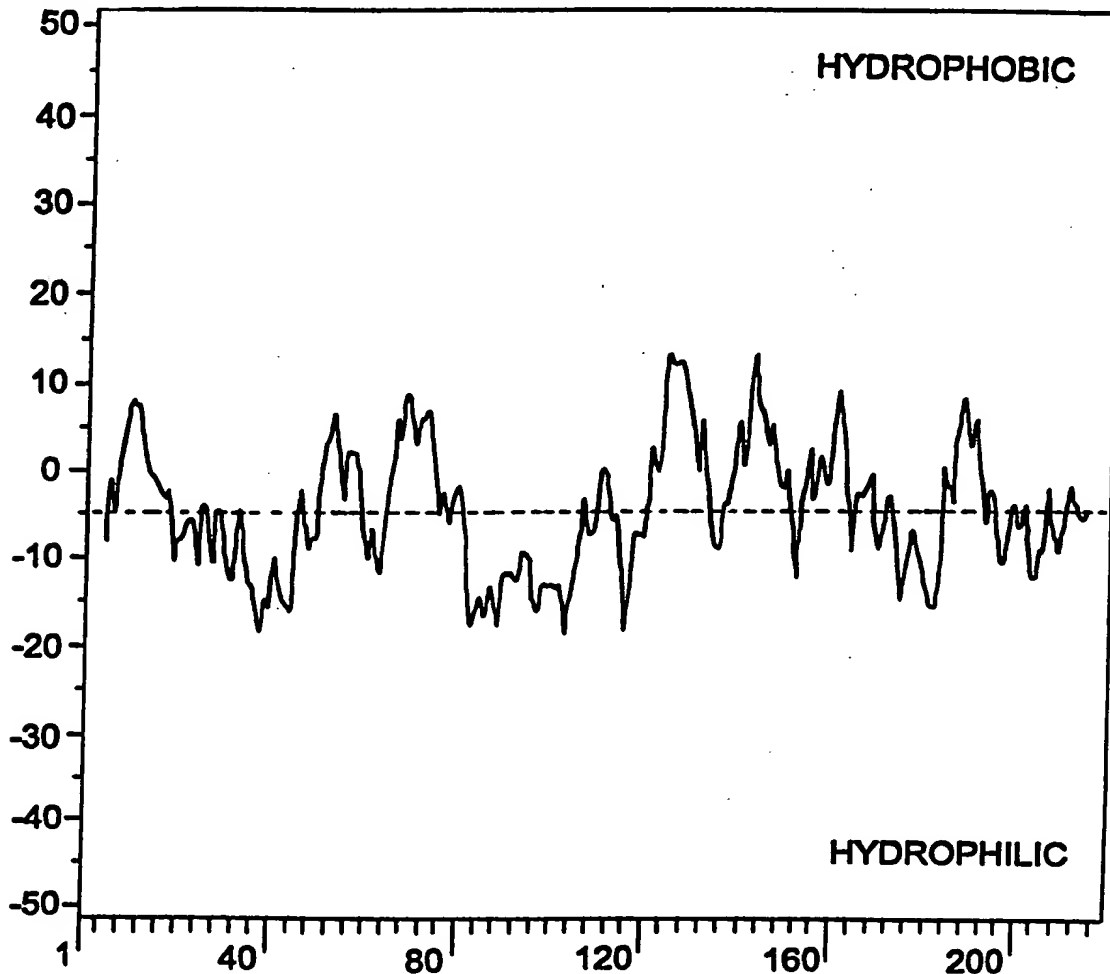COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=-5.31).
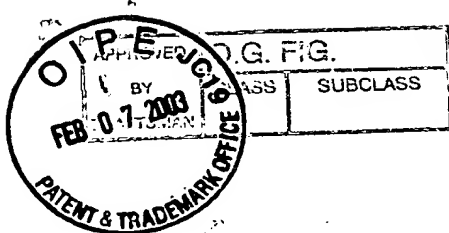
FIG. 3D.

Title: Compositions and Methods for Altering Amino Acid Content of
Proteins
Inventor(s): Rao et al.
Application No: 09/478,598
Atty Dkt No: 5718-16A (35718/193734)

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VSPβ-met10 sequence

<u>SfiI</u>

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1     GGCCCAGCCGGCCAGATCTTCGGAGATGAAATGCGCTAGCTTTAGGCTTGCTGTGGAAGC   60
      CCGGGTCGGCCGGTCTAGAAGCCTCTACTTTACGCGATCGAAATCCGAACGACACCTTCG

61    ACACAACATGCGAGCCTTTAAAACCATTCCTGAAGAGTGCATGGAACCAACAAAGGACTA   120
      TGTGTTGTACGCTCGGAAATTTTGGTAAGGACTTCTCACGTACCTTGGTTGTTTCCTGAT

121   CATGAATGGCGAACAATTTCGAATGGACTCTAAAACAGTTAACCAACAGGCCTTCTTTTA   180
      GTACTTACCGCTTGTTAAAGCTTACCTGAGATTTTGTCAATTGGTTGTCCGGAAGAAAAT

181   TGCTAGTGAAATGGAAATGCATCACAACGACATGTTTATATTCGGCATGGATAACACCAT   240
      ACGATCACTTTACCTTTACGTAGTGTTGCTGTACAAATATAAGCCGTACCTATTGTGGTA

241   GCTCTCTAATATCCCATACTATGAAAAACATGGATATGGGGTGGAGGAATTTAATGAAAC   300
      CGAGAGATTATAGGGTATGATACTTTTTGTACCTATACCCCACCTCCTTAAATTACTTTG

301   CTTATATGATGAATGGGTTAACAAGGGCGACGCACCGGCATTGCCAGAGACTCTTAAAAA   360
      GAATATACTACTTACCCAATTGTTCCCGCTGCGTGGCCGTAACGGTCTCTGAGAATTTTT

361   TTACAACAAGCTGATGTCCCTTGGCTTCAAGATGGTATTCTTGTCAGGAAGGTACCTTGA   420
      AATGTTGTTCGACTACAGGGAACCGAAGTTCTACCATAAGAACAGTCCTTCCATGGAACT

421   CAAAATGGCCGTAACAGAAGCAAACCTAATGAAGGCTGGCTTCCACACATGGGAGCAGTT   480
      GTTTTACCGGCATTGTCTTCGTTTGGATTACTTCCGACCGAAGGTGTGTACCCTCGTCAA

481   AATTCTCAAGGATCCACATCTTATGACTCCAAATGCACTTTCATACAAATCAGCAATGAG   540
      TTAAGAGTTCCTAGGTGTAGAATACTGAGGTTTACGTGAAAGTATGTTTAGTCGTTACTC

541   AGAGAATATGTTGAGGCAGGGATACAGAATTGTTGGAATGATTGGTGATCAATGGAGCGA   600
      TCTCTTATACAACTCCGTCCCTATGTCTTAACAACCTTACTAACCACTAGTTACCTCGCT

601   TCTGCTTGGAGACCACATGGGCGAATCTAGAACCTTTAAGCTTCCTAATCCCATGTACTA   660
      AGACGAACCTCTGGTGTACCCGCTTAGATCTTGGAAATTCGAAGGATTAGGGTACATGAT

661   CATGGAGGCGGCCGC   675
      GTACCTCCGCCGGCG
              NotI
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Fig. 4

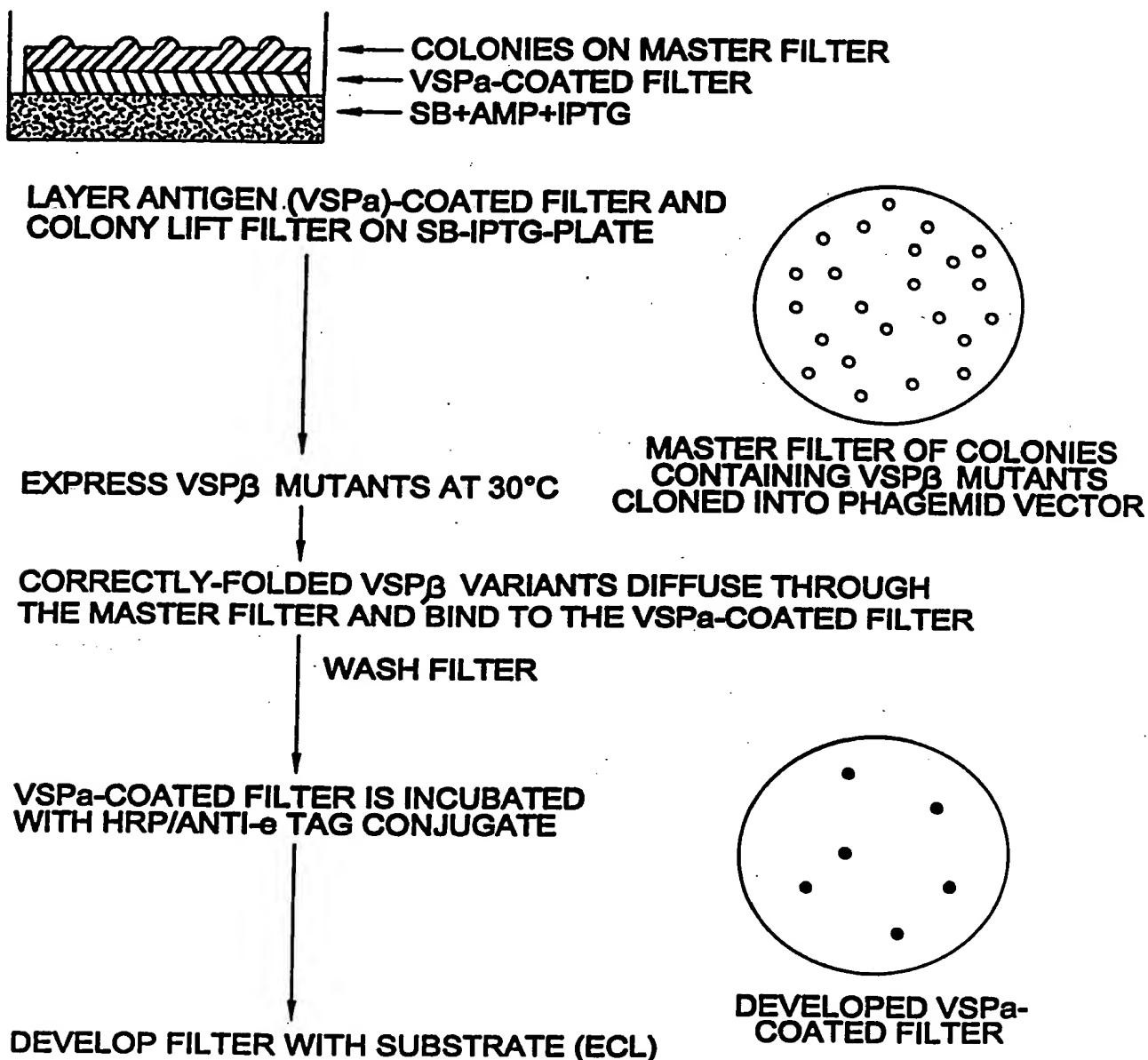## COLONY LIFT ASSAY TO DETECT PROTEIN-PROTEIN INTERACTIONS

— COLONIES ON MASTER FILTER
— VSPa-COATED FILTER
— SB+AMP+IPTG

LAYER ANTIGEN (VSPa)-COATED FILTER AND
COLONY LIFT FILTER ON SB-IPTG-PLATE

MASTER FILTER OF COLONIES
CONTAINING VSPβ MUTANTS
CLONED INTO PHAGEMID VECTOR

EXPRESS VSPβ MUTANTS AT 30°C

CORRECTLY-FOLDED VSPβ VARIANTS DIFFUSE THROUGH
THE MASTER FILTER AND BIND TO THE VSPa-COATED FILTER

WASH FILTER

VSPa-COATED FILTER IS INCUBATED
WITH HRP/ANTI-e TAG CONJUGATE

DEVELOPED VSPa-
COATED FILTER

DEVELOP FILTER WITH SUBSTRATE (ECL)

FIG. 5.